TWO-YEAR STUDY ON THE PREVALENCE OF PROCESSED ANIMAL PROTEINS IN FEEDINGSTUFFS

KRZYSZTOF KWIAZEK, AND ANNA WEINER

Department of Hygiene of Animal Feedingstuffs,
National Veterinary Research Institute, 24-100 Pulawy, Poland
kwiatekk@piwet.pulawy.pl

Received for publication July 18, 2007

Abstract

The purpose of the study was to determine the prevalence of processed animal proteins (PAP) in selected feed materials and feedingstuffs in Poland. For the detection of PAP, a microscopic method was used. In 2005, among 3 085 samples of feedingstuffs, 117 (3.79%) were contaminated by PAP of terrestrial animal origin. In 2006, out of 6 146 samples of feedingstuffs examined, PAP originating from terrestrial animals were detected in 429 (6.98%) samples. The overall percentage of positive samples of feed materials was ranging from 3.52% in 2005 to 9.60% in 2006. The growing tendency in number of positive samples was also observed in compound feedingstuffs for non-ruminants. In 2005 and 2006, the level of contamination of examined compound feedingstuffs reached 5.88 and 8.89%, respectively. On the other hand, a decreasing level of PAP contamination of feedingstuffs for ruminants should be noticed. In 2005, among 862 samples, PAP from terrestrial animals was detected in 9 (1.04%) samples and in 2006, out of 1 436 examined samples only 8 (0.56%) samples were contaminated. It should be pointed out that a microscopic method proved to be a useful and sensitive technique for the detection of PAP in feedingstuffs.

Key words: feedingstuffs, microscopic method, processed animal proteins, Poland.

According to this law, all processed animal proteins are prohibited for feeding food animals, due to the lack of animal species specific detection methods. Some derogations to the feed ban were made, e.g. for certain blood products and hydrolysed proteins (7). There is no established limit for PAP presence in feed, so this means that a zero tolerance is applied.

At present, a ban on feeding farm animals with animal derived proteins is in force in EU (5-7, 23, 24). There are still some possibilities of the occurrence of PAP in feedingstuffs because of cross-contamination during production or illegal use in animal feeding. For these reasons, there is a need to establish the laboratory control programme to monitor presence of not allowed PAP in feedingstuffs (2, 8). The EU legislation defines basic guidelines for the identification of constituents of animal origin in feedingstuffs by microscopic analysis (4). Poland, as a member of EU, has to follow the requirements contained in Community Law (27, 28).

Taking these facts into account, the study was undertaken in the frame of the national official control programme to determine the presence of PAP in feedingstuffs produced in Poland in 2005 and 2006.

Material and Methods

Feed samples. In 2005 and 2006, 3 085 and 6 149 samples of different feedingstuffs were examined, respectively. In this period, there were examined 1 798 samples of feed materials and 7 436 samples of compound feedingstuffs, consisting of 2 298 samples of feedingstuffs for ruminants and 5 138 samples for non-ruminants. The samples were examined within the official control programme in 16 Regional Laboratories and the National Veterinary Research Institute.

All the samples were taken from factories, stores, feed mills, farms, and means of transport by official regional veterinary inspectors.

Detection method. For the detection of PAP, a microscopic method described in the Directive 2003/126/EC of 23 December 2003 on the analytical method for the determination of constituents of animal
Principles of detection. The constituents of animal origin were identified on the basis of typical, microscopically identifiable elements i.e. muscle fibres and other meat particles, cartilages, bones, horns, hairs, bristles, blood, feathers, egg shells, fish bones, and scales. The identification was done on both the sieve fractions and concentrated sediment of the sample.

Introductory preparation of the sample. Fifty grammes of the sample, depending on the nature of the material, were depelleted or ground using the suitable grinding equipment. The representative part of the sample was applied as a thin layer on an object stage and screened thoroughly under a stereomicroscope at 25 and 40 magnifications.

Detection and identification of constituents of animal origin in the concentrated sediment. Five grammes of the sample were weighed with use of suitable balance into a beaker, and in the next step, the sample was treated with 50 ml of tetrachloroethylene. After that, the mixture was shaken repeatedly and left to stand for at least five minutes to allow the sediment to be separated. The sediment was dried in a fume cupboard. The entire dried sediment or its part was examined for characteristic bone constituents under stereomicroscope and biological microscope (17).

Detection and identification of PAP (meat and bone meal – MBM) constituents by the usage of embedding and staining reagents. The microscopic identification of constituents of animal origin was supported by the use of the cystine reagent and paraffin oil. The cystine reagent was prepared in a laboratory in accordance with the rule described in the Directive 2003/126/EC (4). The components of cystine reagent were: 2 g of lead (II) acetate trihydrate pure, 100 ml of distilled water, 2 g of lead (II) acetate trihydrate pure, and 10 g of sodium hydroxide pure, and 10 ml of distilled water. The suspension was heated carefully. During heating, the processed cystine-containing constituents, such as hairs or feathers become black-brown. The paraffin oil served as an embedding agent for the identification of bones. The bone constituents were identified in this embedding agent on the basis of the presence of lacunae filled with air and visible under a microscope as black holes about 5 to 15 µm of diameter (17).

As proved in previous numerous studies, the sensitivity of the microscopic method used was determined on 0.1% level (9-13, 19, 22, 24).

Results

The numbers of the examined samples and detailed results are given in the Table 1.

As shown in the Table 1, overall 1 798 samples of feed materials and 7 436 samples of compound feedingstuffs were examined for the presence of PAP. Among 1 798 samples of feed materials, 114 (6.34%) were found positive and contained PAP from terrestrial animals. Additionally, in 33 (1.84%) out of 1 798 samples of feed materials, PAP of fish origin was detected. Beside the feed materials, compound feedingstuffs for ruminants and non-ruminants were examined for the presence of PAP of terrestrial animal and fish origin. Out of 2 298 samples of compound feedingstuffs for ruminants 17 (0.74%) and 8 (0.35%) samples contained PAP of terrestrial animal and fish origin, respectively. Moreover, as it appears from the data presented in the table, among 5 138 samples of compound feedingstuffs for non-ruminants, the presence of PAP of terrestrial animal and fish origin was detected in 415 (8.08%) and 156 (3.04%) samples, respectively. In all positive samples contaminated by terrestrial animal PAP, most often feathers, hairs, and bones were detected, which had typical microscopic structure (Figs 1, 2, 3, 4).

Table 1

<table>
<thead>
<tr>
<th>Year of the examination</th>
<th>Number of official samples tested for PAP</th>
<th>Total of number of samples tested for PAP</th>
<th>Number of non-compliant samples</th>
<th>Presence of PAP from terrestrial animals (%)</th>
<th>Presence of PAP from fish (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Feed materials for ruminants</td>
<td>Feed materials for non-ruminants</td>
<td>Compound feedingstuffs for ruminants</td>
<td>Compound feedingstuffs for non-ruminants</td>
<td>Feed materials for ruminants</td>
</tr>
<tr>
<td>2005</td>
<td>965</td>
<td>862</td>
<td>1258</td>
<td>3085</td>
<td>34 (3.52)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>74 (5.88)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6 (0.70)</td>
</tr>
<tr>
<td>2006</td>
<td>833</td>
<td>1436</td>
<td>3880</td>
<td>6149</td>
<td>80 (9.60)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>341 (8.89)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 (0.14)</td>
</tr>
<tr>
<td>Total</td>
<td>1798</td>
<td>2298</td>
<td>5138</td>
<td>9234</td>
<td>114 (6.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>415 (8.08)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>8 (0.35)</td>
</tr>
</tbody>
</table>
**Fig. 1.** Feather fragment after staining with cystine reagent (40x).

**Fig. 2.** After careful heating of the sample, cystine reagent stained hair became black coloured (40x).

**Fig. 3.** Fragment of the bone of poultry with holes (lacunae). The lacunae are less elongated (circular or oval) and dense (40x).

**Fig. 4.** Bone fragment of the mammal with holes (lacunae) that are oval to oblong (200x).

**Fig. 5.** Scale fragment with parallel lines (100x).

**Fig. 6.** Fish bones fragment with long, small holes (lacunae) scattered with numerous radiant lines (caniculae) (200x).
In the case of positive samples contaminated by fishmeal, most often fish bones and scales were detected (Figs 5, 6).

It should be pointed out that percentage of samples contaminated with PAP increased in 2006 compared to 2005. In 2005, among 3 085 samples of feedingstuffs, 117 (3.79%) samples were contaminated by terrestrial origin PAP and in 2006, out of 6 146 samples of feedingstuffs, MBM from terrestrial animals was detected in 429 (6.98%) samples. The highest increase in the percentage of contaminated samples was observed in feed materials. It ranged from 3.52% in 2005 to 9.60% in 2006. The increased percentage of positive samples of compound feedingstuffs for non-ruminants should be noticed. In 2005, the level of contamination of these feedingstuffs was 5.88% and in 2006 – 8.89%. This can be a result from the contamination of fish meal, which is allowed to be used and is usually added to compound feedingstuffs for non-ruminants. Moreover, there was observed a decreasing tendency in contamination levels for ruminants in the analysed two-year period. In 2005, among 862 samples, PAP from terrestrial animals was detected in 9 (1.04%) and in 2006, out of 1436 samples, only 8 (0.56%) samples were contaminated. It should be pointed out that there was observed a decrease in the contamination with PAP from fish both in feed materials and in compound feedingstuffs. In 2005, the level of contamination was 6.00%.

In conclusion, since PAP may be present in feedingstuffs for ruminants, the consumption of feed for ruminant contaminated by PAP could be an important factor in the transmission and epidemiology of BSE infection. Presently, when PAP of terrestrial animal origin is not allowed to use in this country in feeding of the other food animals, the most important control measure is to avoid on-line cross-contamination or illegal usage.

References


26. Regulation of the Minister of Agriculture and Rural Development (Poland) of January 30, 2003 concerning the list of feed materials derived from animal tissues allowed to use in farm animal nutrition. Law Gazette No. 32, item 280.

27. Regulation of the Minister of Agriculture and Rural Development (Poland) of December 2, 2004 on methodology of analysis for the determination of nutritive components and feed additives in feed materials, premixes, compound feedingstuffs, and medicated feedingstuffs. Law Gazette No. 271, item 2688.

28. Regulation of the Minister of Agriculture and Rural Development (Poland) of March 6, 2006 on methodology of analysis for the determination of nutritive components and feed additives in feed materials, premixes, compound feedingstuffs, and medicated feedingstuffs. Law Gazette No. 54, item 389.


